Program/Abstract # 227
Etsrp/Etv2 initiates endothelial/endocardial and inhibits myocardial differentiation by two distinct mechanisms in zebrafish embryos
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Previous studies have suggested that embryonic vascular endothelial, endocardial and myocardial lineages originate from multipotential cardiovascular progenitors. However, their existence in vivo has been debated and molecular mechanisms that regulate specification of different cardiovascular lineages are poorly understood. An ETS domain transcription factor Etv2/Etsrp has been recently established as a critical regulator of vascular endothelial differentiation in zebrafish and mouse embryos. In this study, we show that Etsrp functions as a critical factor in the choice of endocardial/endothelial versus myocardial cell fates during zebrafish embryonic development. Expression of multiple endocardial specific markers is absent or greatly reduced in etsrp knockdown or mutant embryos. We show that Etsrp regulates endocardial differentiation by directly inducing endocardial nfatc1 expression. In addition, Etsrp function is required to inhibit myocardial differentiation. In the absence of Etsrp function, etsrp-expressing endothelial/endocardial progenitors differentiate as cardiomyocytes. Furthermore, FoxC1a function and genetic interaction between Foxc1a and Etsrp is required to initiate endocardial development but is dispensable for the inhibition of myocardial differentiation. These results argue that Etsrp initiates endothelial/endocardial and inhibits myocardial differentiation by two distinct mechanisms. Our findings are important for the understanding of genetic pathways controlling cardiovascular differentiation during normal vertebrate development and will also greatly contribute to the stem cell research aimed at regenerating heart tissues.

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Program/Abstract # 228
Zebrafish mutant in Alpha-Cardiac Actin serves as a model for dilated cardiomyopathy
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Heart failure is the primary manifestation of dilated cardiomyopathies, half of which are characterized as idiopathic (IDC), of unknown etiology, which affect 5 to 8 in every 100,000 individuals (Abraham and Bristow, 1997). A subset of patients with hereditary IDC were found to have missense mutations in ACTC (Olson, 1998). Forward genetic screens in zebrafish have led to the unbiased identification of genes involved in important developmental processes. In a chemical mutagenesis screen (Beis, 2005), a cardiovascular-specific mutant was identified that lacks blood circulation, displays blood regurgitation in a dilated heart between the atrium and ventricle and lacks endocardial cushion formation. Through positional cloning techniques, we have identified the mutation as a single nucleotide change in Alpha-Cardiac Actin (actc1a), resulting in an amino acid substitution at residue 169. This Y169C change in actc1a results in an impaired ability of cardiac monomeric actin to polymerize into F-actin. Physiological analysis reveals that actc1a mutant embryos show a defect in contractility, measured by ventricular shortening fraction (VSF) and atrial stroke volume increase. Molecular markers for endocardial cushions including Notch1b, NFATc1 and klf2a are downregulated or mislocalized in actc1a mutants. Our results support a model that hemodynamics plays an important role in the cardiac valve formation. In actc1a mutants, abnormal cardiac blood flow patterns result in endocardial cushion-specific gene misexpression, and the absence of valvular development. Therefore actc1a zebrafish mutant will be an important tool to study the consequence of actc1 mutations in human congenital disorders that are also commonly associated with septal defects.

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Program/Abstract # 229
Impaired heart function in embryos depleted for the voltage-gated calcium channel beta 2 subunit [CACNB2] is due to reduced cardiomyoctye proliferation and adhesion
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Voltage-gated calcium channels (VGCCs) are oligomeric complexes composed of pore-forming CACNA subunits and several auxiliary proteins. Auxiliary CACNB subunits regulate VGCC electrophysiology and chaperone CACNA subunits to the cell membrane. To determine the contributions of CACNB2 to cardiac development, we depleted zebrafish embryos of CACNB2 transcripts using morpholinos. Although heart fields were of normal size initially, by the time of cardiac cone formation, fewer cells expressed cardiac markers. From 24 to 48 hours post-fertilization (hpf), hearts of CACNB2-depleted embryos (morphants) contained up to 30% fewer cells. Hearts of morphants demonstrated significantly fewer BrDU-positive cells, but no change from wildtype in the number of TUNEL-positive cells. Morphant heart tubes had increased expression of BMP4 (an inhibitor of cell proliferation) at 25 and 48 hpf. Therefore, CACNB2 plays an important role in cardiac cell proliferation. In addition, morphant heart tubes fragmented easily when placed under pressure, suggesting that adhesion among cardiomyocytes was weakened. Consistent with this hypothesis, immunohistochemistry showed cadherins at cardiomyocyte membranes were depleted in morphant hearts. Since heart rhythm was normal in CACNB2 morphants, other CACNB proteins expressed in the heart may enable overtly normal VGCC contractile activity. We are currently assaying whether CACNB2 phenotypes are mediated by loss of VGCC function per se, or by loss of other CACNB2:partner interactions. The latter possibility is intriguing in light of recent data suggesting that CACNBs, as MAGUK-family proteins, may interact with multiple protein partners via their SH3 or guanylate kinase domains.

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Program/Abstract # 230
Zebrafish as a model to study cardiomyopathy
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Proper development of the heart is crucial to embryonic and adult survival. Cardiac maturation, both morphologically and physiologi-